STAIN TECHNOLOGY

VOLUME 19 APRIL, 1944 NUMBER 2

DEMONSTRATING THE OSSEOUS SKELETON OF HUMAN EMBRYOS AND FETUSES

CHARLES R. and ELEANOR NOBACK, *Department of Anatomy, University of Minnesota, Minneapolis; and Department of Microanatomy, University of Georgia, School of Medicine, Augusta*

ABSTRACT.—**Transparent human embryos and fetuses whose osseous skeletons are stained in toto by alizarin red S are successfully prepared when the KOH clearing of the soft tissues and the alizarin staining of the bones are performed simultaneously instead of independently. This modification minimizes the possibility of macerating and staining the soft tissues. Fetuses over 50 mm. CR length are skinned, eviscerated, decerebrated, defatted by dissection, fixed in 95% alcohol, bleached in H_2O_2, cleared and stained simultaneously in an aqueous solution of KOH (from 2% to 10% depending upon the size of the specimen) and .0001 to .00005% alizarin red S (solution has a pale lavender color). This solution is changed periodically to maintain the concentration of the KOH until the clearing of the tissues is complete and of the alizarin until the bones are properly stained. Tissues are dehydrated in increasing concentrations of glycerin and stored in white glycerin plus thymol.**

INTRODUCTION AND HISTORY

This is a report of a tissue clearing and a bone staining technic, demonstrating the *in situ* osseous skeleton, in which the KOH clearing and the alizarin red S (sodium 1,2-dihydroxyanthraquinone-3-sulfonate) staining steps of the modification are executed simultaneously instead of independently. The clearing of the soft tissues and the staining of the bones can be effectively controlled and the maceration and the staining of the soft tissues can be easily prevented in this modification. Human embryos and fetuses from 25 mm. crown-rump (CR) length through six fetal months have been successfully cleared and their bones stained red by this method. The fetuses over six lunar months did not clear well in all regions. The problems of fixing, differentially staining, dehydrating, clearing and storing specimens, here omitted, are adequately discussed in the literature.

Lemnius (1581), Belchier (1736) and others (see Cameron, 1930; 1932 and Ercoli and Lewis, 1943) recognized the property of madder to stain bone red. Beale (1858), who prepared the first transparent human embryo in 1853 with NaOH and glycerin, Schultze (1897), who described the KOH method of clearing embryos, and Mall (1906), who introduced Schultze's technic in America, are the pioneers in the strong alkali and glycerin clearing technics. Lundvall, (1905), Dawson (1926), Hollister (1934) and many others have perfected modifications of the technic using KOH for clearing the soft tissues and alizarin red S for staining the bones.

The fixation of the alizarin dye is ascribed to its chemical affinity for recently deposited calcium salts (Macklin, 1917, Cameron, 1930 etc.). However Ercoli and Lewis, (1943) claim that alizarin red S fixation occurs by a process of adsorption (physical phenomenon) between existing calcium phosphate and dye anions, rather than by the supposed formation of calcium alizarate or a reaction with free calcium ions (chemical phenomenon).

Procedure

1. All human fetuses larger than 50 mm. CR are skinned, eviscerated, decerebrated through a slit in the frontal fontanelle and defatted by dissection. Acetone does not effectively remove the fat in large fetuses. Some of the neck, facial, pelvic and other heavy musculature should be removed from the large fetuses. Experience is the only guide as to the amount of dissection required. Embryos and fetuses under 50 mm. CR may be eviscerated. They are fixed in 95% alcohol for two weeks to two months depending on the size of the specimen. As the alizarin red S staining affinity is dependent on the calcium salts in the bone (Cameron, 1930), the decalcifying properties of neutral or slightly acidic formalin may possibly render this preservative unsatisfactory for the alizarin staining of slightly calcified bony structures.

2. Material is bleached in a mixture of 9 volumes of 95% alcohol to one volume of 3% aqueous H_2O_2, and returned to 95% alcohol until ready for clearing.

3. Fetuses are cleared and stained *simultaneously* in a solution of alizarin red S[1] in aqueous KOH (preferably in distilled water; see Hollister, 1934). The concentration of the dye should be 0.0001 to 0.00005% (which has a pale lavender color); and the strength of the KOH should be varied as follows:

[1]Alizarin red S, used in this technic, was alizarine technical which was obtained from George T. Walker Co. Minneapolis, Minnesota.

a. For fetuses up to 150 mm. CR length, 2 to 4% KOH.
b. For fetuses over 150 mm. CR length, 4 to 10% KOH.

The solutions are changed periodically in order to maintain the concentration of KOH and the alizarin red S in the solutions until the tissues are adequately cleared and the bones are well stained. Adequate clearing (bones clearly visible) may take from 3 to 4 days in small specimens to 2 to 3 weeks for six-month fetuses. When the bones are adequately stained, if clearing is not complete, it may be continued in an aqueous solution of KOH or in a dilute glycerin solution (10% glycerin in distilled water) of KOH.

In previously published technics the specimens are first cleared and then the bones are stained in a solution of alizarin red S in KOH. While staining the skeleton in this solution, the extraosseous tissues continue to clear. If the clearing of a specimen has been carried too far during the clearing step of the technic, maceration of the soft tissues often results while the bones are staining. In this modification the process of clearing is completed at the same time as the staining of the bones, or slightly later, and thus the clearing step can be stopped at the proper time and yet have the bones stained intensely red. When low concentrations of alizarin red S are used for staining the bones, the bones stain intensely red and the extraosseous tissues do not stain at all. Thus the clearing of the soft tissues and the staining of the bones can be effectively controlled, and the maceration and the staining of the soft tissues can be easily prevented.

4. Specimens are finally cleared and dehydrated in increasing concentrations of glycerin. Specimens, cleared in glycerin, are easily dissected, do not undergo shrinkage, keep indefinitely and clear completely.

5. For storage, white glycerin, in which a crystal of thymol is dissolved to prevent mold from growing, is used. Specimens should be stored in darkness although some exposure to light does not visibly destain the bones.

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VARIOUS OIL SOLUBLE DYES AS FAT STAINS IN THE SUPERSATURATED ISOPROPANOL TECHNIC

R. D. LILLIE, *Senior Surgeon, U.S.P.H.S., The Division of Pathology, National Institute of Health, Bethesda, Md.*

SUMMARY.—**Oil red O (xylene-azo-xylene-azo-β-naphthol), oil red 4B or EGN (xylene-azo-toluene-azo-β-naphthol) and Sudan red 4B give somewhat deeper orange red or red fat stains and more stable dilute isopropanol solutions than Sudan IV. Sudan II gives brighter orange-yellow fat stains and stronger stable dilute isopropanol solutions than Sudan III. Satisfactory brownish red dyes as to intensity and stability of their dilute isopropanol solutions are Sudan brown, Sudan brown 5B, and oil brown D.**

Following previously reported work[1] on the relative efficiency of a number of oil soluble dyes as fat stains when used in dilute acetone methanol solutions, Lillie and Ashburn[2] reported on the use of supersaturated solutions in 50 to 60% isopropanol as fat stains. In that report favorable comments were made on oil red 4B and Sudan brown as substitutes for Sudan IV.

Previously I had accepted the identification of oil red O as *m*-xylene-azo-β-naphthol, given under No. 73 in the Colour Index[3] and accepted by Conn[4], but preferred the name Sudan II for this dye. The dye so named in the first report[1] was obtained from the Commonwealth Color and Chemical Company and from the Hartman Leddon Company, both of whom have identified the samples they furnished as C.I. No. 73 or Sudan II, though the first company named their sample brilliant oil scarlet B concentrated, while the second called both of theirs oil red O. National Aniline Division has recently furnished another sample of Sudan II (C.I. No. 73) under the name oil scarlet 6G, with the definite assurance that it is C.I. No. 73.

The National Aniline Division now gives the information that the dye supplied by *them* as oil red O is not, and never has been C.I. No.

[1]LILLIE, R. D. (In press.) Study of certain oil soluble dyes for use as fat stains. J. Tech. Methods; accepted September 1, 1942.

[2]LILLIE, R. D., and ASHBURN, L. L. 1943. Supersaturated solutions of fat stains in dilute isopropanol for demonstration of acute fatty degenerations not shown by Herxheimer technic. Arch. Path., **36**, 432–5.

[3]ROWE, R. M. 1924. Colour Index. Soc. Dyers and Colourists, Bradford, Yorkshire.

[4]CONN, H. J. 1940. Biological Stains, *4th Ed.* Biotech Publications, Geneva, N. Y.

73, and that the statement in the Colour Index that their oil red O was a synonym of Sudan II is erroneous. Actually their oil red O is a disazo dye similar to Sudan IV, namely *p*-xylene-azo-*p*-xylene-azo-β-naphthol and even more similar to the oil red 4B mentioned above, which is *p*-xylene-azo-*o*-toluene-azo-β-naphthol. This latter dye is also sold by the National Aniline Division as oil red EGN.

Since French[5] specified in his original report that the oil red O used by him was obtained from National Aniline and that it had a greater depth of color than Sudan III (which Sudan II has not), it is evident that the dye referred to was *p*-xylene-azo-*p*-xylene-azo-β-naphthol and not C.I. No. 73.

The purpose of the present report is to compare a number of oil soluble dyes with Sudan IV, using the new supersaturated isopropanol technic[6]; 35 samples of oil soluble dyes were used, tabulated in Table 1. Saturated stock solutions of all were made in 99% isopropanol, using 200 to 500 mg. per 100 ml. For use all were diluted to 60% by addition of 4 ml. water to 6 ml. stock solution. These dilutions were allowed to stand 5 to 10 minutes, then filtered, and, if further turbidity or precipitation appeared in the first half hour, they were again filtered. Frozen sections of adrenal cortex were stained 10 minutes in these dilutions during the period between 30 minutes and 4 hours after dilution, and another series 24 hours or more after dilution. When fat staining was poor, other sections were given an hour's exposure to the stain. After the fat stain all sections were transferred to water and counterstained 5 minutes in acid hemalum,[7] which had been diluted with 4 vol. of 2% acetic acid. (This is the same concentration as Mayer's original formula, undiluted.) They were then blued in 1% aqueous Na_2HPO_4, rinsed and mounted in modified Apathy syrup. Results are presented in Table 1. There are also given certain spectroscopic data serving to help identify the samples. The value d $\times$ D (i.e. dilution times optical density at the absorption maximum) is inserted as a crude measure of relative solubility in 99% isopropanol. It must be regarded as a crude measure, owing to the variations in composition of the various dyes.

In general, pleasing deep orange red to red colors are given by the Sudan IV, oil red 4B and EGN, oil red O, Sudan red BB and Sudan red 4B. Some of the Sudan IV samples show rather rapid precipitation of the dye and consequent impairment of staining; others appear to give fairly stable solutions. The others of this group have some-

[5]French, R. W. 1926. Fat stains. Stain Techn., **1**, 79.

[6]See footnote 2.

[7]Lillie, R. D. 1942. An improved acid hemalum formula. Stain Techn., **17**, 89–90.

TABLE I. SPECTROSCOPIC AND STAINING DATA ON VARIOUS OIL SOLUBLE DYES.

C.I. NO.	NAME, MANUFACTURER AND LOT NUMBER	λ	W	m	R	d·D	COLOR OF FAT	GRADE OF 10′ STAIN FRESH	GRADE OF 10′ STAIN DAY OLD	REMARKS
73	SUDAN II, CO#26871	498	41	498	1.42	187	ORANGE YELLOW	+++	++	+++ 20′
73	" HL (A)	494	40	498	1.40	226	" "	+++	++	+++ 20′
73	" HL (B)	496	41	498	1.40	168	" "	+++	++	+++ 20′
73	" NA#10547	494	41	496	1.55	456	" "	++++	+++	
81	SUDAN BROWN, NIH#6.942	492	94	477	1.20	39	DEEP RED BROWN	++++	+++	
82	NAPHTHYLAMIN BORDEAUX, NIH#5.2242	491	60	481	1.39	22	ORANGE BROWN	++	+	
113	SUDAN R, NA#9317	500	40	504	1.22	109	ORANGE YELLOW	++	++	
248	SUDAN III, CB#CY-5	504	39	508	1.12	13	BRIGHT ORANGE	+	+	
248	" CB#CY-6	508	37	508	1.14	26	" "	++	++	
248	" GR	506	42	508	1.12	48	" "	+++	+++	
248	" KY-1	512	44	510	1.07	24	" "	++	+	
248	" NA#NY-6	512	40	510	1.08	16	" "	+++	+++	
248	" NA#NY-7	502	35	502	1.30	17	" "	+++	+++	
248	" NIH#11.2643	505	36	504	1.24	5	ORANGE	±	±	+ 1hour
258	SUDAN IV, CB#CZ-5	512	39	512	1.04	50	ORANGE RED	+++	±	+ 20′
258	" CB#CZ-7	521	41	519	0.96	26	" "	+++	−	∓ 20′
258	" HL#LZ-3	512	37	513	1.04	61	" "	+++	++	
258	" HL#LZ-4	514	35	514	1.03	53	" "	+++	+++	
258	" NA#NZ-11	514	39	517	1.01	37	" "	+++	±	+ 20′
258	" NA#NZ-13	516	39	518	0.97	46	" "	+++	++	
	OIL RED 4B, CO#26870	513	43	515	1.03	64	DEEP ORANGE RED	+++	++	
	OIL RED EGN, NA#123063	522	37	521	0.93	31	DEEP RED	+++	+	
	" NA#165113	518	29	515	0.99	68	" "	++++	++	
	OIL RED O, NA#BC40105	516	38	517	0.97	128	" "	+++	++	+++ 20′
	" NA#10517	521	37	515	0.99	63	" "	++++	+++	+++ 20′
	" NA#BC40104	521	36	518	0.97	57	DEEP ORANGE RED	++++	++++	
	OIL BROWN D, NA#104960	522	63	514	0.99	46	DEEP RED BROWN	++++	++++	
	OIL BROWN M, NA#9259	490	53	487	1.60	35	BROWN	++	++	
	OIL BROWN Y, NA#7356	454	48	446	2.23	142	BROWN ORANGE	++	++	
F.P. NO.	AZO OIL BLACK B, NA#116739	568	57	568		28	DEEP BLUISH RED	±	±	+ 1hour
177	SUDAN BLUE G, GD#S0.049	640	21	639		8	BLUE GREEN	∓	−	
178	SUDAN BROWN 5B, GD#S0.49	478	56	482	1.31	53	BROWN RED	++++	++++	
182	SUDAN RED BB, GD#S.0.049	515	37	517	0.99	14	RED	++	+++	
	SUDAN RED 4B, GD#S0.049	517	39	518	0.96	50	"	++++	++++	
	SUDAN CORINTH B, GD#S0.49	520	63	519	0.98	51	BROWN RED	+++	++++	

C.I. NO. = COLOUR INDEX NO., F.P.NO. = FOREIGN PROTOTYPE NO., λ = ABSORPTION MAXIMUM IN mμ., W = WIDTH IN mμ OF ABSORPTION BAND OF OVER 90% MAXIMUM DENSITY(D)., m = MEDIAN OF FOREGOING ABSORPTION BAND, R = RATIO: D500/D530 = CONN'S E500/E530, d·D = DILUTION (d) X DENSITY (D) AT λ OF SATURATED ISOPROPANOL SOLUTION DILUTED IN 95% ETHANOL, ′ = MINUTES. CO = COMMONWEALTH COLOR AND CHEMICAL CO., HL = HARTMAN LEDDON CO., NA = NATIONAL ANILINE DIVISION, NIH = NATIONAL INSTITUTE OF HEALTH, CB = COLEMAN AND BELL CO., GR = GRUEBLER, GD = GENERAL DYESTUFFS CORP.

what deeper orange red or purer red colors, and the 60% isopropanol solutions still stain well 24 hours after dilution. The solubility of Sudan III in isopropanol appears to be too low to give consistently good stains, but the solutions appear to be fairly stable after dilution. However, if a lighter orange yellow stain is desired, for instance to contrast with the blue-black myelin of the Weigert iron hematoxylin method, Sudan II gives good solubility, quite stable dilute isopropanol solutions, and a sharp bright orange yellow color. The color tone of the browns is not pleasing to some observers, but Sudan brown, Sudan brown 5 B (foreign prototype No. 178 in the Yearbook of the American Association of Textile Chemists and Colorists) and oil brown D (constitution not published) give satisfactory color intensities and sharp staining. Sudan R, naphthylamine bordeaux, oil brown M, oil brown Y, and Sudan corinth B give orange or brown tones of lesser intensity and brilliance, though their dilute isopropanol solutions still stain well after 24 hours. Azo oil black B gives good color but requires prolonged staining, while Sudan blue G and Victoria blue B base were unsatisfactory.

Since Sudan III showed variation in color from orange to orange red in various samples, and concurrent variation in position of the spectroscopic absorption band from a median of 501.9 to 510.4 mμ (Sudan IV ranged from 512.0 to 518.7), it was determined to synthesize a sample from the purest available aminoazobenzene and β-naphthol. The resultant sample was apparently less soluble in isopropanol than any of the commercial samples and gave an absorption band median at 504.0 mμ. It is probable that the redder commercial samples are more or less contaminated with the homologous aminoazotoluene derivative.

Acknowledgment is due to Medical Technician Roland Faulkner for technical assistance.

MICROSCOPY WITH PLASTIC SUBSTITUTES FOR COVER GLASSES

OSCAR W. RICHARDS, JOHN H. SMALL and P. WARDHAM COLLYER
Research Department, Spencer Lens Co., Buffalo, N. Y.

ABSTRACT.—**To determine whether plastic substitutes for cover glasses on microscope slides affect the performance of the microscope, their optical constants were determined. The plastic covers and glass cover glasses were mounted also on silvered slides to form Star Test Plates which were studied by competent observers. The thicker plastic cover glasses, now on the market, are satisfactory when mounted to give a plane surface. Optically inhomogeneous materials, of irregular thickness, those that curl or do not have plane surfaces, adversely affect the performance of the microscope and should not be used. Since the substitutes are softer than glass they must be protected from abrasion. It is recommended that thicknesses of 0.18 mm., and none outside of a range of 0.12 to 0.20 mm. be used. For critical observation with unimmersed objectives of high aperture, best results are obtained with the correction collar set at the position corresponding to the actual thickness of the cover slip, just as would be done with glass cover glasses.**

Plastic sheeting has been recommended as an inexpensive substitute for glass cover slips in microscopic study. The suitability of these substitutes from a chemical and mechanical viewpoint has been described. This paper reports on the affect of these materials on the performance of the microscope.

Little information on the optical properties of the plastic covers is available.[1] Microscopists have reported their use with varying satisfaction. Kenohan (1928) used moisture proof Cellophane (DuPont) 0.25 mm. thick with Wright's medium. Suntzeff and Smith (1940, 1941) recommended VuePack (Monsanto) 0.17 mm. thick for mounts with balsam and isobutyl methacrylate, but withdrew their recommendation on finding that their preparations faded. Cellophane 0.022 mm. thick was preferred to an isobutyl methacrylate film by Bucholtz (1940).

Prevost (1942) used Cellophane mounts sealed with petrolatum for temporary preparations for dark field examination. Cellophane cover slips (No. 88 gauge) cut from rolls 7/8" wide were found satisfactory by Pons (1942) after nine months. Tissues imbedded in

[1]See Pfund (1939) and Strain *et al.* (1939).

No. 120 Cellophane, or Nos. 005, 0125 and 020 VuePak, were handled conveniently as a ribbon by Minckler (1942). Russell (1942) reported VuePack inexpensive but unsuited for permanent mounts. Elias (1942) found plastic sheets helpful when handling large numbers of sections.

A thin film of plastic evaporated onto a preparation has been used instead of a cover slip. Such films are too thin to be satisfactory optically, or to be tested in this study, although they do provide mechanical protection. Microscope objectives corrected for use without a cover glass should be used for best results on preparations covered with such thin films.

TABLE 1.—OPTICAL CHARACTERISTICS OF GLASS AND PLASTIC COVER GLASSES

Material	N_D	Thickness t mm.	t/N_D	Correction Collar Setting	
				(I)	(II)
Cellulose Acetate A	1.488	0.14	0.094	13	13
Cellulose Acetate B	1.491	0.18	0.121	18	18
VuePak	1.498	0.13	0.087	13	12
Cellophane	1.535	0.03†	0.020	*u*	*u*
Vinylite A	1.528	0.16	0.105	16	17
Vinylite B	1.528	0.14	0.092	(15)*	(13)*
Pliofilm	1.54	0.04†	0.026	*u*	*u*
Glass A	1.5140	0.19	0.125	19	19
Glass B	1.5249	0.17	0.111	17	18
Glass C	1.5281	0.14	0.092	14	14

t/N_D = equivalent optical path in air.
u = Below limit of correction collar.
† = Surface wavy and irregular.
* = Inhomogeneous optically, varies from region to region.

Lucite was not found to be a satisfactory mounting medium as it faded stained preparations (Richards and Smith, 1938). Styrene (R38, Dow) and Vinylite (National Carbon and Carbide) also caused fading, but Petrex (Hercules) dissolved in methyl salicylate may be useful.[2] O'Brien and Hance (1940) recommended an isobutyl methacrylate film, and a dibutyl phthalate polystyrene mixture was used by Lendrum and Kirkpatrick (1942). Polyvinyl alcohol has been suggested recently by Downs (1943).

Samples of commonly used plastic cover glasses were obtained and their refractive indices (n) measured. From the measured thickness (t) of the material the equivalent air path (t/n) was computed for each and recorded in the table. The plastic covers, and samples of

[2]Personal communication (1939) from J. A. Smith, then of Johns Hopkins University.

glass cover glasses from different manufacturers were mounted with Clarite on a silvered slide of proper thickness to form a Star Test Plate. The silver film has small holes, which, when properly illuminated by the microscope condenser, become point sources. From the position, shape and color of the images of these small points of light the skilled observer can assess the aberrations in the optical system; see Belling (1930).

Two competent observers (I and II) studied the star images and determined the best setting for the correction collar on a Spencer 4 mm. N.A. 0.95 apochromatic objective used with high eyepoint compensating eyepieces. The observations are recorded in the table. The very thin samples of Cellophane and Pliofilm had such short equivalent air paths that it was not possible to correct for the spherical aberration by means of the correction collar. The irregular surfaces also contributed to the blurred images that were seen with the microscope. Such thin materials should never be used on preparations for critical examination.

The other materials listed in the table were thick enough to give optical paths long enough to permit correction for their actual thickness with the adjusting collar of the objective. Plotting the equivalent air paths against the observed correction collar settings showed fairly close agreement for both the plastic substitutes and the glass cover slips with about the same amount of variation in each group. To obtain the best image with the microscope for critical work the correction collar of the objective should be set to the value that corresponds with the actual thickness of the cover slip, just as with glass cover slips.

The Vinylite, and a commercial plastic cover slip presumably Vinylite, judging from its optical constants, developed a brownish coloration of the silver film of the test plate within a few days. Some of this material was also optically inhomogeneous and gave various values for different regions; average values were placed within parentheses in the table. While the refractive index is nearer that of glass, Vinylite may not be satisfactory unless optically homogeneous sheets can be selected.

The dispersion values, showing the relative effect on light of different colors, of the plastics are lower than those for the glass cover slips, but neither observer saw any marked chromatic aberration with the thicker plastic samples. The differences are probably too small to give noticeable color aberration with sheets thin enough to be suitable for microscope cover glasses.

Conclusions

Examination of plastic substitutes for use as cover slips for preparations to be examined with a microscope showed that the thicker ones now on the market are satisfactory when mounted to give a plane surface. Materials optically inhomogeneous, of irregular thickness, that curl or give irregular surfaces, adversely affect the performance of the microscope and should not be used. As the substitutes are softer than glass they must be protected from abrasion. It is recommended that thicknesses of 0.18 mm., and none outside of a range of 0.12 to 0.20 mm. be used. For critical observation with unimmersed objectives of high aperture, the correction collar should be set, for best results, to the position corresponding to the measured thickness of the cover slip, just as would be done with glass cover glasses.

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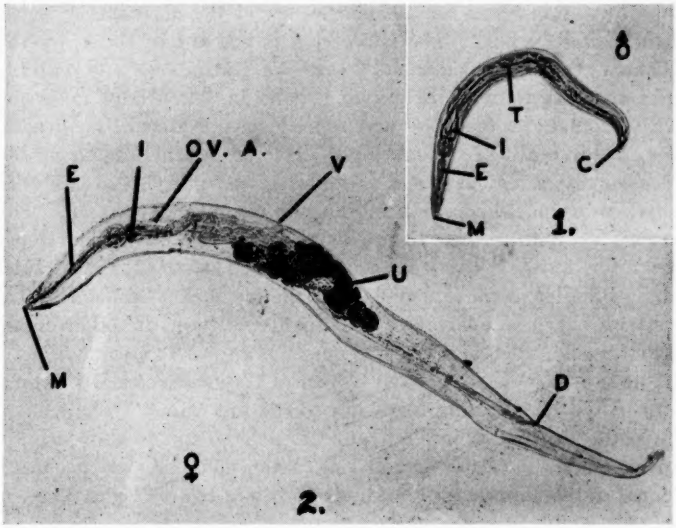
RAPID CLEARING OF PIN WORMS (*ENTEROBIUS VERMICULARIS*) FOR CLASS STUDY

DONA MINCKLER, *Department of Microanatomy, St. Louis University School of Medicine, St. Louis, Missouri*

ABSTRACT.—**Pin worms can be cleared and differentiated in one operation after formalin fixation and dioxane dehydration by using carbol xylene. The entire procedure, including fixation time, requires less than 3 days. The mounts are permanent and give sufficient detail for class study.**



Enterobius vermicularis. 1, male; 2, female.

Explanation of symbols: m, mouth; e, esophagus; i, intestine; t, testis; c, cloaca; ov. a., anterior ovary; v, vagina; u, uterus; d, anus.

Although pin worms are readily available to most laboratories and present no diagnostic problem, they have been found difficult to prepare for class study. The following technic has been found to clear pin worms successfully, leaving good differentiation between internal organs and tissues of the body wall, yielding sufficient detail for class work:

1. Fix in 10% formalin.
2. Wash out formalin in running water, several hours.

3. Dioxane, 3 changes, 1 hour each.
4. Carbol xylene (25 ml. phenol to 75 ml. xylene), 24 hours.
5. Three changes of neutral xylene, a few minutes each.
6. Mount in damar dissolved in xylene and cover.

The worms are handled in a small round bottle. Successive liquids are decanted, and since the worms adhere to the glass, the bottle is thoroughly drained each time by setting it mouth down on filter paper. Individual worms are mounted from the last xylene by using a large caliber medicine dropper.

The body wall is colored a light tan and is translucent. In the anterior end, the prominent digestive organs are a darker tan outlined by a thin black line, while the rest of the digestive system is slightly more yellow. The nerve ring is not differentiated by this technic. When partly distended with ova, the anterior and posterior uteri are dark brown. In gravid females in which huge masses of ova surround the digestive and reproductive systems, the ova are identified in outline only, leaving the colored internal organs visible. Occasionally, a few large ova do not clear, possibly because of their inner, embryonic, lipoidal membrane for chemical protection. This opacity serves to distinguish pre-larval forms from the less developed ova. The anterior ovary is seen as a coiled, striated, yellow tube, often extending as far cephalad as the middle of the esophagus. The posterior ovary is not so easily distinguished from the other organs in that region. Anal and vaginal openings appear as invaginations of the body wall. The digestive system of the male is clearly defined both by color and a thin black line for its full extent. The testis is the same shade of yellow but appears pebbly and is also outlined, Unretouched photomicrographs of male and female specimens. cleared in the manner described, are shown in figures 1 and 2.

NOTES ON TECHNIC

A Modification of the Wirtz Spore-Staining Technic

The modification described here is an outgrowth of attempts to eliminate prolonged heating of dyes on slides.

A heavy suspension, of the organism to be stained, is made in 2–3 drops of water in a 3 × ⅜ inch test tube. An equal quantity of 5% aqueous malachite green is added and the tube is then placed in a boiling water bath for 15–20 minutes.

Smears are made from this stained preparation by spreading a loopful on a clean glass slide, in the usual manner. The smear is allowed to air-dry and when thoroughly dry is fixed by flaming. The prepared smear is decolorized in running tap water for 10 seconds and then counterstained for 1 minute with 0.5% aqueous safranin. The slide is washed again and blotted dry.

This technic stains spores green and vegetative cells red. It has been used on eight species of spore-formers: *Bacillus terminalis*, *Bacillus laterosporus*, *Bacillus alvei*, *Bacillus subtilus*, *Bacillus niger*, *Bacillus vulgatus*, *Bacillus cereus*, and *Bacillus megatherium*, and has been found to give consistently good results in all.

Slides prepared in this manner are much clearer than those made by heating dye directly on the slide. Furthermore, this method has additional advantages: 1) it is easily used, with excellent results, even by inexperienced workers such as undergraduate students; 2) it eliminates the possibility of precipitates, resulting from overheating slides; 3) it consumes much smaller quantities of dye.—Jennie E. Shapiro, *Biology Department, Brooklyn College, Brooklyn, N. Y.*

Anilin Blue as a Counterstain in Cytology

In plant cytology, the usual counterstain used after safranin is fast green F.C.F., or sometimes light green SF yellowish.

When a blue counterstain is desired, anilin blue W.S. is used by some cytologists. Johansen's[1] technic is usually employed, which calls for 2 to 24 hours or even 48 hours in a 1% solution of safranin followed by a saturated solution of the counterstain, the solvent for each dye being equal parts of methyl cellosolve and alcohol with subsequent addition of an equal volume of clove oil to the counterstain. This procedure requires long staining, as well as an unusual solvent.

[1]Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., New York. See p. 82.

The following quick procedure is proposed for the study of chromosomes when using root tips fixed in the CRAF fluid: Remove paraffin from the sections in the usual manner, running down through the alcohols to water. Stain 15 minutes in 1% aqueous safranin 0 and rinse in distilled water. Apply few drops of 1.0% anilin blue W.S. in 95% alcohol[2] for 2 minutes. Dehydrate in absolute alcohol, clear in xylene and mount in balsam.

This gives an excellent picture of cell division and permits microscopic study to be made within 25 minutes. The chromosomes and nuclei are red, the cell walls blue, and the cytoplasm nearly colorless.

Root tips fixed in the specified fluid were found to show sharply stained chromosomes and blue cell walls. The results with Flemming's fluid were not so good, as the contrast between the two dyes was less pronounced, especially in the younger cells at the very tip of the root. With Karpechenko's fixative, good results were obtained by increasing the staining time in alcoholic anilin blue from 2 to 8 minutes.—MARY A. DARROW, *Biological Stain Commission, Geneva, N. Y.*

[2]This dye is sparingly soluble in alcohol, and it is not at all sure that as much as 1.0% can be brought into solution. Experience, however, shows that the results obtained are much inferior if solutions of 0.5% or less are used. The solution should be allowed to stand over night to be sure all the dye possible goes into solution.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

DYES AND THEIR BIOLOGICAL USES

KUHNS, D. M., and FELDMAN, H. A. **Laboratory methods used in determining the value of sulfadiazine as a mass prophylactic agent against meningococcic infections.** *Amer. J. Pub. Health*, **33**, 1461–5. 1943.

Surveys for meningococcus carriers must be made before using mass treatment of such carriers with sulfadiazine as a method for controlling epidemics of this disease. A routine which employs dehydrated media, a simple candle jar, and a rapid serological technic is described for facilitating studies for meningococcus carriers. Swabbings of the nasopharynx are streaked onto chocolate agar plates containing para-aminobenzoic acid, which are incubated in candle jars. Suspicious colonies are subcultured to other chocolate agar plates. Pure cultures from the latter are inoculated into fermentation media, and agglutination tests also are run. The fermentation media have been found extremely effective for both meningococci and gonococci, the consistency of the reactions obtained establishing them as valuable adjuncts for the final identification of Neisseria. Reactions may usually be read in 12 hr. or less in the case of meningococci, while the gonococci may require 48 hr. Spinal fluids may be inoculated directly into the media. Because of its agar content, the medium contains so little oxygen that incubation in the candle jar usually is unnecessary. Three sugars are used routinely: dextrose, maltose, and sucrose. All strains of meningococcus ferment dextrose and maltose, but not sucrose. However, the fermentation of these two sugars may be slow in an occasional strain. The basal medium consists of Bacto-tryptose, 20 g.; NaCl, 5 g.; agar, 1 g.; phenol red (0.2% solution), 0.4 ml.; distilled H_2O to 1000 ml. It is adjusted to pH 7.4 and 5 g. of dextrose, maltose or sucrose are added to 1 liter of medium. It should be sterilized for not more than 10 min. at 10 lb. pressure.—*M. W. Jennison.*

ANIMAL MICROTECHNIC

CLAVERDON, M. ARDELLE. **A new fixative for animal tissues.** *Science*, **97**, 168. 1943.

The following formula is recommended for a superior general fixative: picric acid, 5 parts; isopropanol, 55 parts; acetone, 30 parts; glacial acetic acid, 5 parts; formaldehyde, (40% by vol., C. P.) 5 parts. Fixation, depending on size and nature of tissues, requires 2 hr. to 4 days. After fixation the tissue is washed in nearly absolute isopropanol 1 to 2 hr., followed by three changes of dioxane, 1 to 3 hr. in each change. Infiltration is begun in ⅓ dioxane, ⅔ paraffin, and completed in three changes of pure paraffin, ½ to 1 hr. for each in vacuum oven. Tissues are sectioned at 7 μ and picric acid is removed from the mounted sections with 1.5% NH_4OH in 95% ethanol prior to staining.—*T. M. McMillion.*

COPLEY, A. L., and ROBB, T. P. **Studies on Platelets. I. The method of Vilarino and Pimentel and a new direct method of counting blood platelets.** *Amer. J. Clin. Path.*, **12**, 362–71. 1942.

The authors have tested the method of Vilarino and Pimentel (Klin. Woch., **18**, 1252–6, 1939) for counting platelets, and present a simpler, more direct method using a differential staining technic. The procedure recommended is as follows: Draw 1 ml. of a modified Aynaud solution (0.75% NaCl, 3.8% sodium citrate and 3.7% formaldehyde) into a 2 ml. syringe; then by venipuncture draw exactly 1 ml. more of blood into the syringe, and mix the blood with the solution by including a small air bubble and inverting the syringe 5 times. This blood dilution may be kept 24 hr. at room temperature in a stoppered tube before using. To make the count, invert the tube containing the blood dilution 10 times,

and add 0.5 ml. of the mixture to 12.0 ml. of 3.8% sodium citrate solution; mix 0.5 ml. of this second dilution with 0.5 ml. of a 0.2% solution of brilliant cresyl blue in 3.8% sodium citrate and place 1 drop of the final dilution in a counting chamber. The red blood cells usually fade after 10 minutes and the platelets appear as dark blue bodies. The few red blood cells that take the stain are differentiated from the platelets by their size and shape. The method of Vilarino and Pimentel was found to be reliable but much more involved and time-consuming than the present one.—*L. Farber.*

ESSEX, H. E., and DE REZENDE, NILSON. **Observations on injury and repair of peripheral nerves.** *Amer. J. Physiol.*, **140**, 107–14. 1943.

For *in vivo* studies of the repair of the injured posterior auricular nerve of the rabbit, the authors made use of a transparent window, and injected methylene blue into an artery or vein. The effective concentrations of the dye that stained the nerve distinctly and appeared to be tolerated without serious untoward effects were 2.5 to 5% solutions of methylene blue in physiologic saline solution. Concentrations of 15 to 20% caused considerable edema of the whole ear. Staining at the time of injury and subsequently permitted study of the injury and repair of the nerve.—*Elbert C. Cole.*

FULLER, DOROTHEA. **Two new methods of staining vaginal smears.** *J. Lab. & Clin. Med.*, **28**, 1475–6. 1943.

The following stains are suggested for staining vaginal secretions in studying the menstrual cycle.

Stain I, for routine smears: Fix smears immediately in 95% alcohol and ether (equal parts) for 5 min., run through graded alcohols (80, 50, 30%) to water. Stain in Ehrlich's hematoxylin diluted one half with distilled water for 2 min. Wash in running water for 4 min. Stain 2 min. in solution A (xylidine ponceau 1 g., distilled water 99 ml., and glacial acetic acid 1 ml., added to an equal volume of acid fuchsin 1 g., distilled water 99 ml. and glacial acetic acid 1 ml.). Rinse in water. Stain 2 min. in solution B (orange G 2 g., phosphomolybdic acid 1 g. and distilled water 100 ml.). Superficial cells stain green, deep cells a slightly darker green, highly cornified cells orange, and less cornified cells pink. Fresh red blood corpuscles stain pink, old ones green.

Stain II, for malignant cells: Fix, run down through alcohols to water, and stain in diluted Ehrlich's hematoxylin as in Stain I. Wash and run through ascending alcohols. Stain 2 min. in a mixture of 95% alcohol saturated with eosin, 11 ml., alcohol saturated with orange G, 35 ml., and alcohol saturated with fast green, 8 ml.). Differentiate with alcohol containing 1% glacial acetic acid for 1 min. with agitation. Rinse in absolute alcohol, then xylene, and mount. This stain does not keep well when mixed. Superficial cells stain green, cornified cells red, and malignant cells are usually red or purple.—*John T. Myers.*

LEBLOND, C. P. **Localization of newly administered iodine in the thyroid gland as indicated by radio-iodine.** *J. Anat.*, **77**, 149–53. 1943.

Guinea pigs and rats were injected with the 8-day isotope I^{131} and dogs and rabbits with isotope I^{130}. In all of the animals except dogs, some ordinary chemical iodine (I^{127}) was added as a carrier. In all cases the iodine was given as a single injection.

At various intervals after injection, the animals were killed and two pieces of thyroid taken from each. One was prepared by ordinary paraffin technic, the other by a modified freezing-drying method after the Altmann-Gersch method, freezing in acetone-dry-ice mixture, drying in a Pyrex test tube connected with a Hyvac pump, and refrigerated at −20° C. Paraffin present in the test tube was melted, thus embedding the tissue, and spreading was done without use of water.

The slides were then put on the sensitive face of medium lantern slide plates and left for several days. Each accumulation of radio-iodine was found to affect the photographic plate. After the plate was fixed and developed, the section was stained with hematoxylin-eosin, or Masson's trichrome stain. The developed plates, or "autographs", show the iodine concentrated in the denser appearing eosinophilic colloid, less abundant in the thinner colloid, and absent outside of the follicles.—*Warren Andrew.*

LILLIE, R. D. **Blood and malaria parasite staining with eosin azure methylene blue methods.** *Amer. J. Pub. Health*, **33**, 948–51. 1943.

This article is a brief discussion of some of the factors involved in the manufacture of satisfactory, reproducible Giemsa and Wright stains for blood and parasite work. Spectrographic analysis and control is essential after standardization of the chemical procedures. Quite satisfactory Giemsa and Wright stains are now available. The absorption spectra correlate well with the staining capacity of the dye mixtures. Stains of the Wright type should have an initial absorption maximum between 650 and 660 mμ.—*M. W. Jennison.*

LILLIE, R. D. **Some experiments on the Romanovsky staining of blood films.** *J. Lab. & Clin. Med.*, **28**, 1872–5. 1943.

Wright stain solutions, made up in methanol in the usual concentration of 125 mg. per 100 ml., will give blood and parasite stains comparable to Giemsa stain in color effects, if the dilutions with buffered water are increased from 1:2 to 1:5 or 1:10, and the time increased to 15–30 min. Previous fixation of the films with 80–100% methanol and staining with premixed dilutions of either Wright or Giemsa stain is the preferable procedure. Addition of 5–10% of acetone to Giemsa stain decreases the staining time by about one-half.—*John T. Myers.*

MCMANUS, J. F. A. **A rapid silver stain for nerve-fibers in formol-fixed paraffin sections of the human spinal cord and medulla.** *J. Path. & Bact.*, **55**, 503–5. 1943.

The following method for rapid silver staining of human spinal cord and medulla has the advantage of giving good results with a relatively short period of impregnation: Fix in formol or formol-saline; dehydrate; cut sections 4–6μ thick; fix sections to slide with egg albumin; and bring down to water. Place slides in chloral-hydrate-silver solution at 58° C. for 30–36 min. (chloral hydrate, 1 g.; $AgNO_3$, 20 g. or less; water to 100 ml.) Rinse in several changes of distilled water and reduce in freshly prepared Bodian's solution (hydroquinone, 1 g.; Na_2SO_3, anhyd., 1 g.; distilled water, 100 ml.) for 10 min. Wash in distilled water and treat for 10 min. with 1% aqueous gold chloride containing 4 drops glacial acetic acid per 100 ml. Wash carefully in distilled water. Reduce in 2% aqueous oxalic acid until fibers are visible under the microscope. (The sections are usually gun-metal gray viewed against a white background.) Wash in distilled water and tap water (a few seconds in each). Place in 5% $Na_2S_2O_3$+5 H_2O for 5–10 min. Wash well in tap water. Dehydrate in alcohol, pass through xylene and mount in balsam. Counterstain if desired. For the central nervous system incubate the sections at 58° C. for 20–30 min. in 0.6% NaOH and wash thoroughly before placing the sections in Bodian's solution.—*S. H. Hutner.*

MENDELOFF, J., and BLECHMAN, H. **A combined elastica-trichrome stain for tissues.** *Amer. J. Clin. Path.*, Tech. Sect., **7**, 65. 1943.

Masson's trichrome stain (Amer. J. Path., **4**, 181, 1928) has been combined with Weigert's elastic tissue stain (Mallory, F. P., Pathological Technique. W. B. Saunders, Philadelphia. 1938. See pp. 168–9) in a technic staining elastic tissue blue-black, smooth muscle red, and collagen green. The procedure is as follows: Wash deparaffinized sections thoroughly in water; stain 60 min. in resorcin-fuchsin mixture (no details given); wash rapidly in acid alcohol; dehydrate and differentiate in absolute alcohol until sections are faintly red; pass rapidly through 70% alcohol to water; stain 8 min. in Harris' hematoxylin and immerse 5 min. in water; stain 5 min. in ponceau-acid-fuchsin-orange-G solution (no details given); wash thoroughly in water, and treat 10 min. with 3% phosphotungstic acid solution; wash thoroughly in water, and treat 5 min. with light green solution (no details given); followed by 3 min. in 1% acetic acid; dehydrate, clear, and mount in gum damar.—*L. Farber.*

WORLEY, LEONARD G., and WORLEY, ELIZABETH K. **Studies of supravitally stained Golgi apparatus. I. Its cycle in the tectibranch mollusc, Navanax inermis (Cooper).** *J. Morph.*, **73**, 365–99. 1943.

Eggs of this mollusc were immersed in dilute methylene blue (National Aniline) in sea water, first at a dilution of about 1:1,000,000, then in one of 1:750,000, later

in 1:500,000, and so on, thus gradually, over a period of 3 or 4 hours, bringing them into a 1:100,000 dilution. These operations were carried on in subdued light, and the embryos subsequently kept in semi-darkness to avoid possible photodynamic effects. When properly employed, the methylene blue stained the Golgi elements of mollusc eggs dark blue, the mitochondria pale blue-green or not at all, while the fatty and albuminous yolk appeared pale yellow-green. The methylene blue was not toxic in the dilutions employed.—*Elbert C. Cole*

PLANT MICROTECHNIC

ARMITAGE, F. D. **Further uses for chlorazol black E and a new stain for botanical sections.** *J. Roy. Micr. Soc.*, **63**, 14–19. 1943.

To a mixture of phenol crystals, 2 parts by weight, lactic acid, 2 parts by weight, glycerin, 1 part by weight, water, 2 parts by weight, sufficient chlorazol black E is added to give the solution the appearance of India ink. After this has stood for a few days, fungal hyphae and sporangia gradually take up the dye and are easily differentiated into their component parts.

For permanent mounts, the fungus, on Czapek agar, is fixed by flooding the culture dish with 70% alcohol. After at least 3 hr. a few ml. of a saturated solution of chlorazol black E in 70% alcohol are added. When the staining is sufficient, the stain is poured off and the culture is dehydrated with alcohol. A portion is cut out and mounted on a slide in a generous amount of euparal. A cover slip is added. Alternatively, the specimen may be dehydrated in dioxane and mounted in a mixture of dioxane, gum sandarac, and camsal.

To stain paper fibers, about 0.5 g. of paper is boiled 1 min. in saturated aqueous chlorazol black E. The excess dye is removed by running water. The fibers are disintegrated by forming a pellet which is rubbed between finger and thumb; then it is boiled in a tube of distilled water and shaken vigorously. A small portion is pipetted on to a clean slide, which is dried on a hot plate at 70° C. Canada balsam or euparal is used as mountant. A better but slower method begins by disintegrating and boiling the fibers as before, then transferring them to a slide where they remain adherent. They are stained by chlorazol black E in 70% alcohol for 30 min. or longer, then dehydrated and mounted in balsam or euparal. Samples of heavily coated paper must be boiled in 5% aqueous KOH, then thoroughly washed before staining.

Differential staining of sections of plant parts is obtained by the following procedure:

Sections of material fresh from the field are placed in 70% alcohol or in a mixture of 5 ml. 40% formaldehyde, 7 ml. glacial acetic, and 90 ml. 70% alcohol. After at least 15 min. (storage in the fixative may be continued several days), the sections are brought down to water and left at least 8 hr. in a mixture of equal parts of 6% $MgSO_4$ and saturated aqueous chlorazol azurine G 200, after heating the mixture to 80° C. and stirring it well. The mixture remains useful for 2 or 3 days after its preparation. After staining, sections are passed quickly through the alcohols to absolute and are mounted in euparal.

Satisfactory staining of sections of herbarium material has been obtained by soaking or boiling in water, then passing them directly into the mixture of $MgSO_4$ and chlorazol azurine G 200.—*C. E. Allen.*

SHEFFIELD, F. M. L. **Value of phloem necrosis in the diagnosis of potato leaf-roll.** *Ann. Appl. Biol.*, **30**, 131–6. 1943.

Potato leaf-roll is a virus disease whose diagnosis in the field is not easy. In affected plants a necrosis of the primary phloem in the bicollateral bundles occurs which is distinct from any abnormality produced by any other cause. The following technic is suggested for demonstrating phloem necrosis: take a piece from the base of a main stem including about six nodes; trim off leaves and branches; cut stem into pieces of suitable length for hand sectioning; place pieces in 50% alcohol; cut sections by hand through nodes; place sections in phloroglucinol solution (1% in 50% alcohol) on a slide; drain after 1 min.; place in 50% HCl for 1 min.; drain; mount in water and examine at magnification of about × 100. In the healthy plant, most of the phloem will be colorless

but fibers may be pink or red; in the infected plant, some primary phloem strands will be yellowish red. If leaf-roll virus is present, some of the six nodes will show phloem necrosis.—*H. P. Riley.*

MICROÖRGANISMS

BERNHARDT, ERNEST. **A simplified method for staining fungi in skin and nails.** *Arch. Dermat. & Syph.*, **48**, 533–5. 1943.

The author recommends the following procedure: Place on a slide a small particle of the material (not exceeding $2 \times 2 \times 0.2$ mm.). Macerate in 10% KOH. Cover. Remove excess alkali by firm pressure under blotting paper. (Maceration is certainly incomplete if the specimen tends to slip out or if air invades the covered space.) Flood the specimen with a drop of cotton-blue-lactophenol and heat it gently, blotting off excess stain from the opposite rim. Then press it firmly under blotting paper. Microscopic examination shows the objects on a slightly bluish background. Flooding the specimen with clear lactophenol removes the last traces of excess stain. Lactophenol is composed of 1 part each of phenol crystals, lactic acid syrup and water and 2 parts glycerin. The staining solution is made up by adding 0.5% of cotton blue Poirrer to the lactophenol, dissolving and filtering. Specimens sealed with Noyer's cement did not show appreciable fading after 1 year of storage.—*J. A. Kennedy.*

GOLDSMITH, J. B. **A differential stain for the demonstration of Trichinella larvae in tissue.** *Trans. Amer. Micr. Soc.*, **62**, 327–8. 1943.

Good contrast between muscle and *Trichinella* larvae either encysted or in migration was secured as follows: Fix in Bouin's fluid overnight; rinse in water or 50% alcohol to remove excess fixative but not picric acid color; stain in bulk in Delafield's or Harris' hematoxylin overnight; destain in acid alcohol until all hematoxylin color has been removed from cytoplasm; alkalize in alkaline ($LiCO_3$ saturated) alcohol. Embed in celloidin or paraffin. Results: muscle fibers, yellow with picric acid; nuclei of muscle fibers and cells of cyst walls, purple; larvae of *Trichinella*, bluish to purple.—*Virgene Kavanagh.*

KRAJIAN, A. A. **A new and dependable method for the demonstration of acid-fast organisms in tissue sections.** *Amer. J. Clin. Path., Tech. Sect.*, **7**, 45–8. 1943.

The author has modified the acid-fast stain by changing the type of nuclear stain and decolorizer, and by introducing a differentiating step. The method is applicable to frozen and paraffin sections fixed in the usual solutions. The procedure is as follows: dehydrate frozen sections, 7–10 μ, blot in filter paper, dip once in celloidin and allow to dry on the glass slide; wash in tap water until surface is wet; gently steam 3 min. with carbol-fuchsin; wash in tap water and treat with arsenic acid alcohol (1% arsenic acid in 60% alcohol), with constant agitation of slide until most of the red color is removed; wash in tap water and treat 2 min. with Loeffler's methylene blue; wash in tap water; dehydrate with 3 treatments of anhydrous ethanol or isopropanol; treat at once 2 or 3 times with isocreosote (equal parts of anhydrous ethanol or isopropanol and beechwood creosote), agitating slide constantly for an even differentiation; blot immediately in filter paper; clear in 3 changes of xylene of 1 min. each and mount in gum damar. Paraffin sections are deparaffinated with 2 changes of xylene of 2 min. each and 2 changes of anhydrous ethanol or isopropanol, washed in tap water, and then treated as frozen sections.—*L. Farber.*

PACKER, R. A. **The use of sodium azide (NaN_3) and crystal violet in a selective medium for streptococci and Erysipelothrix.** *J. Bact.*, **46**, 343–9. 1943.

A selective medium using NaN_3 and crystal violet as inhibiting agents is able to eliminate much of the difficulty in culturing streptococci and *Erysipelothrix rhusiopathiae* from contaminated materials. These bacteria can be inhibited selectively by adding varying amounts of these two agents to a basal medium of tryptone broth or agar. Stock solutions of 0.25 g. crystal violet (94% dye content) in 100 ml. distilled water and of 1 g. NaN_3 in 25 ml. distilled water were used to make the dilutions in the broth or agar.—*Virgene Kavanagh.*

PERRIN, T. G. **A stain for Treponema pallidum.** *Amer. J. Clin. Path. Tech. Sect.*, **7**, 28. 1943.

The staining procedure recommended is as follows: Prepare smears on slides from exudate obtained by compressing base of chancre or by scraping surface of ulcer; allow to dry in air or fix in flame; stain 2 min. with heat, or 6 min. at room temperature in a mixture of Ziehl's carbol-fuchsin 4 ml., acetic acid 1 ml., commercial formalin 1 ml., and distilled water 10 ml.; wash by gentle agitation in water and dry in air.—*L. Farber.*

YEGIAN, D., and BUDD, V. **Ziehl-Neelsen technique. Staining properties modified by different preparations of basic fuchsin.** *Amer. Rev. Tuberc.*, **48**, 54. 1943.

Yegian and Baisden (J. Bact., **44**, 667, 1942) have shown that the staining properties of the tubercle bacillus of the acetate and chloride salts of the possible components of basic fuchsin (rosaniline and pararosaniline) are quite different; acetate salts of the dyes give smears with a large percentage of beaded organisms, whereas relatively few beaded bacilli are found with the chloride salts. It was also observed that a final washing of the stained preparation in 95% ethanol gives smears with bacilli solidly stained, regardless of the dye salt used. The present report is a study to determine which salt of fuchsin is most suited for the demonstration of the tubercle bacillus.

Solutions of pararosaniline acetate and chloride and rosaniline chloride (National Aniline and Coleman & Bell) in 95% ethanol were used. Cultures were washed free of media and suspended in distilled water before being stained. A platinum loopful of the aqueous suspension was spread on a slide and dried at room temperature. The dye solution was then flooded on the slide and steamed gently for 5 min. After cooling for 3–5 min. at room temperature, the dye was drained off, the slide thoroughly washed with water, decolorized with 5% HCl in 95% ethanol for 90 sec.; washed with water and counterstained 30 sec. with brilliant green (1% in 0.01% NaOH).

It was found that the composition of the dye markedly affected the appearance of the tubercle bacillus. Saturated solutions of the acetate salts gave largely beaded smears, whereas weaker solutions gave faintly stained smears with few or no beaded forms. The chlorides of the dyes in saturated solutions gave smears with relatively few beaded organisms; 0.3% solutions gave smears with no beaded organisms and all the bacilli were solidly stained and easily recognized.

The authors recommend that 0.3% solutions of the chloride salts be used in the Ziehl-Neelsen staining technic and that the indefinite term "basic fuchsin" be discontinued, giving instead the exact composition of the dye on the label, such as "Rosaniline Chloride".—*L. Farber.*

BY-LAWS
OF
BIOLOGICAL STAIN COMMISSION, INC.[1]

ARTICLE I

NAME, LOCATION, OBJECTS, ETC.

1. The name of this corporation shall be the "BIOLOGICAL STAIN COMMISSION, INC."

2. The purposes for which said corporation is to be formed are:

(a) The standardization of biological stains, that is dyes or products and preparations of dyes used for any and all purposes in laboratory procedures;

(b) To carry on investigations looking towards the perfecting of the supply of biological stains, and towards the development of new uses for them;

(c) To publish scientific data relating to the nature and use of biological stains;

(d) To issue statements of certification to manufacturers which they may use in accordance with and subject to the rules and regulations from time to time adopted by the Board of Trustees of this organization on different batches of stain that have been tested by the Commission;

(e) To carry on investigations in related fields wherever they seem desirable towards bringing about any of the objects of this organization;

(f) This organization shall take over the duties, rights and privileges of the unincorporated organization known as the Commission on Standardization of Biological Stains, and to own and to dispose of such property as is now being held by the latter.

This corporation is not organized for profit, and no part of the net earnings shall inure to the benefit of any member of the corporation or individual.

3. The principal office of this corporation shall be in the City of Geneva, County of Ontario and State of New York.

4. The seal of this corporation shall have inscribed upon it the words, "Biological Stain Commission, Inc. Corporate Seal 1943."

ARTICLE II

MEMBERSHIP

1. The members shall be members of the Commission on the Standardization of Biological Stains at the time of incorporation, together with such new members as may be elected from time to time by the Board of Trustees. Said members shall remain in good standing as long as annual dues, imposed by the Board of Trustees, are paid. Members shall be entitled to reduced rates or other privileges in connection with Commission publications as may be decided upon from time to time by the Board of Trustees. The Board of Trustees may, in its discretion, fix dues for membership.

[1]These by-laws were adopted at a meeting held in Philadelphia, Feb. 21, 1944, to incorporate and to accept a charter granted to the Biological Stain Commission by the University of the State of New York, as a non-profit corporation. The meeting was attended by six members of the Executive Committee of the Commission on Standardization of Biological Stains, which now becomes the Biological Stain Commission, Inc. The following officers were elected: President, H. J. Conn; Vice-president, W. F. Windle; Secretary, Louis Gershenfeld; Treasurer, S. I. Kornhauser. These four officers constitute the Board of Trustees of the new organization, with the following additional trustees: E. V. Cowdry, J. C. Hinsey, R. D. Lillie, W. J. Robbins, J. T. Scanlan, A. E. Severinghaus, W. D. Stovall.

2. Any national scientific society interested in biological stains may request representation on the Commission. If approval is granted by the Board of Trustees, said society may designate a representative to the Commission. This representative is to be known as an accredited delegate member.

3. Every member shall be entitled to one vote at any meeting of the corporation.

4. Members shall have no personal right, title or interest in or to any of the property and assets of this corporation.

Article III

MEETINGS

1. The annual meeting of this corporation for the election of trustees and for the transaction of such other business as shall properly come before it, shall be held in each year at such time and place as may be determined upon from time to time by the president of this corporation.

2. Special meetings of this corporation may be called at any time by the President, or if he cannot function, by the Vice-president, and if the President and Vice-president are unable to function, the duties of such office shall be assumed by the Secretary, and if the latter cannot function by the Treasurer. Special meetings may also be called upon the written demand of three or more trustees.

3. Notice of meetings, written or printed, for every regular or special meeting of this corporation, shall be prepared and mailed to the last known postoffice address of each member not less than ten days before any such meeting, and if for a special meeting, such notice shall state the object or objects of such meeting. No failure or irregularity of notice of the annual meeting shall invalidate the same or any proceeding thereat.

4. Five members shall constitute a quorum of this corporation for the transaction of business at any meeting, but a less number by a majority vote of those present, may adjourn any meeting from time to time, without notice other than by announcement at the meeting until a quorum be present. Any meeting at which a quorum is present may also be adjourned in like manner by a majority of the members present. At any adjourned meeting at which a quorum shall be present, any business may be transacted which might have been transacted at the original meeting.

5. The order of business at the annual meeting, and as far as possible at all meetings, shall be:

(a) Calling of roll.
(b) Proof of due notice of meeting.
(c) Reading and disposing of any unapproved minutes.
(d) Reports of officers and committee.
(e) Election of trustees.
(f) Unfinished business.
(g) New business.
(h) Adjournment.

6. Any member may at any time waive, in writing, any notice required to be given under these By-Laws.

7. The President, if present, shall preside at all meetings of the corporation and Board of Trustees. In his absence the next officer in due order, who may be present, shall preside. For the purposes of these By-Laws the due order of officers shall be as follows: President, Vice-president, Secretary and Treasurer.

8. The record and proceedings of any meeting of this corporation may be ratified by any member by ratification in writing.

9. At all meetings of this corporation all questions, excepting the question of amendment to these By-Laws, and such other questions, the manner of deciding which is established or regulated by statute or by other special provision of these By-Laws, shall be determined by a majority vote of the members present provided, however, that a quorum must be present.

Article IV

Board of Trustees

1. The governing power and management of all property, affairs and business of this corporation, and all powers of this corporation, except as otherwise provided, shall be vested in a board of eleven trustees. The eight members of the Executive Committee of the Commission on Standardization of Biological Stains at the time of incorporation, shall become the initial members of the Board of Trustees, and these eight members shall hold office until the election of their successors. They shall convene after the incorporation and elect the three additional members to the Board of Trustees, whose term of office shall be for one year. When any of the following organizations, namely:

Society of American Bacteriologists
American Medical Association
American Association of Anatomists
American Association of Pathologists and Bacteriologists
American Chemical Society
American Public Health Association
American Pharmaceutical Association
American Society of Zoologists

appoints a new representative to the Commission, the new representative does not, however, become automatically a member of the Board of Trustees. The former representative of that Society shall continue to serve as a member of the Board of Trustees until the next meeting or the first occasion thereafter when elections are being held. On such occasion the members present shall, by a majority vote, fill the vacancy created. A majority of the Board must always be Society representatives.

2. Meetings of the Board of Trustees shall be held at such times and places as may be decided upon by vote of said Board from time to time.

3. Special meetings of the Board of Trustees may be called at any time by the President and in the event of his being unable to function then by the Vice-president. Any three members of the board may request in writing a "Special Meeting" of the board. Said request is to be addressed to the President.

4. Five members of the Board of Trustees shall constitute a quorum for the transaction of business at any meeting, but a less number, by a majority vote of trustees present, may adjourn any meeting from time to time without any notice other than by announcement at the meeting until a quorum be present. Any meeting at which a quorum is present may also be adjourned in a like manner by a majority of the trustees present. At any adjourned meeting at which a quorum shall be present, any business may be transacted which might have been transacted at the original meeting.

5. The officers of this corporation shall be elected in each year by the Board of Trustees from the membership of said Board at their first meeting after the annual meeting of this corporation and they shall hold office for one year next ensuing, or until their successors shall be duly elected and qualified. The Board of Trustees shall have power at any meeting to fill any vacancies.

6. The order of business at any regular or special meeting of the Board of Trustees shall be:

(a) Reading and disposal of any unapproved minutes.
(b) Reports of officers and committees.
(c) Election of officers.
(d) Unfinished business.
(e) New business.
(f) Adjournment.

7. Proceedings of less than a quorum of the Board of Trustees may be ratified by any or all of said trustees, but said ratification must be in writing.

8. The affairs of this corporation between meetings of its Board of Trustees shall be managed by an Executive Committee of three, consisting of the President, the Secretary and Treasurer of this corporation. In the event of the offices of secretary and treasurer being held by the same person, then the other member of the Executive Committee shall be the Vice-president. The executive committee shall have all the powers of the Board of Trustees when it is not in session.

Article V

Officers

1. The officers of this corporation shall be President, Vice-president, Treasurer and Secretary. Of these the Treasurer may hold the office of vice-president or of secretary, if such combination of officers seems desirable to the Board.

2. The initial officers of this corporation shall be those holding the same office in the Commission for Standardization of Biological Stains at the time of the incorporation. They shall hold office until resignation or until a successor is appointed.

3. The President shall preside at all meetings of the corporation and at all meetings of the Board of Trustees; shall sign all contracts and other instruments of this corporation requiring his signature, or as he may be authorized from time to time by the corporation or the Board of Trustees; shall make a report to the annual meeting of this corporation and shall perform such other duties as are incident to his office or are properly required of him by the corporation or the Board of Trustees.

4. The Treasurer shall, by virtue of his office and during his tenure thereof, be the custodian of all funds, securities, evidences of indebtedness, moneys and other property belonging to the corporation. In case of the absence or inability of the Treasurer to perform his duties as treasurer of this corporation, or in case of a vacancy, his powers and duties as treasurer of this corporation shall be delegated by the Board of Trustees to any other member of the Board until such time as the Treasurer is able to act and perform his duties, or until his successor is duly elected and qualified to act. The Treasurer shall keep regular books of account; he shall perform all duties incident to his office or that are properly required of him by the corporation or the Board of Trustees; shall make a final report to the annual meeting of this corporation and shall give bond at the expense of the corporation for the faithful performance of his duties in such sum and with such sureties as may be required from time to time by vote of the Board of Trustees.

5. The Secretary shall issue notice of all meetings of the corporation and Board of Trustees and shall keep minutes thereof. He shall have custody and charge of the corporate seal and other corporate books, papers and documents, except as otherwise provided in these By-Laws, or otherwise directed by the Board of Trustees; shall make such reports and perform such other duties as are incident to his office or are properly required of him by the corporation or the Board of Trustees.

6. In case of the absence or disability of any officer of this corporation or for any other reason that may be deemed sufficient by the Board of Trustees, the Board may delegate his powers and duties for the time being to any other officer or to any member of the Board of Trustees.

7. If at any time it becomes desirable to hold a regular or special meeting of the Board of Trustees for the election of any officer and it cannot be arranged promptly, a member or two members of the said Board of Trustees shall be nominated as a candidate or as candidates for the office in question. Within two weeks of said nomination ballots shall be mailed to all members of the Board of Trustees at their last known address, on which shall be printed or written the name of the candidate or candidates nominated by the Board of Trustees, and the blank space for writing in the name of any other member of said Board for which a vote is to be cast instead of for a candidate nominated by said Committee. The polls shall be open from the day of mailing said ballot until ten o'clock P.M. on the day of election, as specified on the ballots. The said day of election shall be at least two weeks after the date of mailing the ballots. The ballots shall be opened by tellers selected by the Board of Trustees, who shall declare the person receiving the largest number of votes cast to be elected. The officer thus elected shall take office immediately.

8. There shall also be an Editor of STAIN TECHNOLOGY, which office may be held by any one of the other officers and whose duties shall be those indicated in Article VII, Section 2. Said Editor shall be elected in the same manner as the officers except that a candidate for the office need not be a member of the Board of Trustees. If not a member of said Board at the time of his election, he shall become one *ex officio* upon election.

ARTICLE VI

FINANCE

1. The moneys and all valuable effects and securities of this corporation shall be deposited in the name of this corporation in such depositories as the Board of Trustees may designate. The Board of Trustees shall have charge of investing the funds of this corporation. No funds, income or moneys of this corporation shall be invested or re-invested in any securities or property except such securities and property as are authorized as legal investments for Savings Banks by the laws of the State of New York at the time said investment is made; provided, however, that any investments or securities turned over to this corporation by the Commission on Standardization of Biological Stains may be retained or reinvested along with any other income received therefrom from time to time in like securities, in the absolute discretion of the Board of Trustees. No investments or reinvestments of the funds or moneys of this corporation shall be made except upon the authority of the vote of the Board of Trustees or its Finance Committee.

2. A Finance Committee shall be elected by the Executive Committee with the President of the corporation a member ex officio. This committee shall have all the powers of the Board of Trustees with respect to handling of investments and other financial matters between meetings of the Board.

3. All checks, drafts and orders for the payment of money shall be signed by any two of the following officers—the President, Vice-president, Treasurer, Secretary; except that special accounts of limited amounts for purposes specified by the executive committee may be established from which checks may be drawn signed by either the President or Treasurer.

4. The Treasurer or President may endorse for collection or deposit all drafts and checks which are in terms made payable to or are intended to be paid to the Commission.

5. The books and accounts of the Treasurer of this corporation shall be audited by a committee to be appointed by the Board of Trustees.

Article VII

PUBLICATIONS

1. The official publication of this corporation shall be STAIN TECHNOLOGY. The President, with the approval of the Board of Trustees, shall have the right to arrange for the Commission's sponsorship of other publications relating to the subjects in which the Commission is interested.

2. STAIN TECHNOLOGY shall be in charge of the Editor who shall be responsible for the editorial policies and business management of the publication, and shall have the right to appoint an editorial board; he may delegate specific duties in connection with the editing or publishing of STAIN TECHNOLOGY to any member of said board. The editor shall not commit STAIN TECHNOLOGY or the corporation to any financial obligation without the approval of the Executive Committee.

Article VIII

AMENDMENTS

1. These By-Laws may be amended, repealed or altered, in whole or in part, by a majority vote of the entire membership of this corporation after having first been approved by the Executive Committee.

2. Voting upon amendments shall be by ballot and may be conducted by mail, in which event the proposed amendment, with ballot, shall be mailed to all enrolled members at their last known address not less than six weeks prior to the date upon the ballot vote is to be taken, which date shall be specified in the ballot and the polls shall be open from the day of mailing the proposed amendment and ballot until ten o'clock P.M. of the day set for the taking of the vote. The ballots cast shall be counted by a teller selected by the Executive Committee. The teller shall render a report to the Board of Trustees and declare the amendment either carried or not carried, as the case may be, and a copy of the teller's report shall thereupon immediately be mailed to every enrolled member of the corporation at his last known address.

BIOLOGICAL STAIN COMMISSION
INITIAL MEMBERSHIP (1944)

BOARD OF TRUSTEES

Officers

President, **H. J. Conn,** New York State Agricultural Experiment Station, Geneva, N. Y. (*representing the Society of American Bacteriologists.*)

Vice-President, **W. F. Windle,** Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. (*American Association of Anatomists.*)

Secretary, **Louis Gershenfeld,** Philadelphia College of Pharmacy and Science, Philadelphia, Pa. (*American Pharmaceutical Association.*)

Treasurer, **S. I. Kornhauser,** University of Louisville Medical School, Louisville, Ky. (*American Society of Zoologists.*)

Other Delegate Members

Cowdry, E. V., Washington University School of Medicine, 4580 Scott Ave., St Louis, Mo. (representing the *American Medical Association.*)

Lillie, R. D., National Institute of Health, Bethesda, Md. (*American Association of Pathologists and Bacteriologists.*)

Scanlan, J. T., Eastern Regional Research Laboratory, Philadelphia (18), Pa. (*American Chemical Society.*)

Stovall, W. D., State Laboratory of Hygiene, Service Memorial Institute, Madison, Wis. (*American Public Health Association.*)

Newly Elected Trustees

Hinsey, J. C., Cornell University Medical College, York Ave. and 69 St., New York, N. Y.

Robbins, W. J., New York Botanical Garden, New York, N. Y.

Severinghaus, A. E., College of Physicians and Surgeons, 630 W. 168 St., New York, N. Y.

Members

Allen, C. E., Biology Bldg., University of Wisconsin, Madison, Wis.

Beams, H. W., Dept. of Zoology, State University of Iowa, Iowa City, Ia.

Beltran, Enrique, Apartado Postal 1079, Mexico, D. F.

Brentzel, Wanda W., State College Station, Fargo, N. D.

Burke, Victor, State College, Pullman, Wash.

Chandler, Clyde, formerly New York Botanical Garden, Bronx Park, New York, N. Y.

Chapman, George H., 604 Fifth Ave., New York, N. Y.

Cole, E. C., Williams College, Williamstown, Mass.

Constable, Francis W., McCloskey General Hopital, Temple, Texas.

Davenport, H. A., Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.

Dawson, Alden B., Biological Laboratories, Harvard University, Cambridge, Mass.

De Tomasi, James A., Bausch & Lomb Optical Co., Rochester, N. Y.

Dutton, L. O., 615 Mills Bldg., El Paso, Texas.

Faull, J. H., Arnold Arboretum, Jamaica Plain, Boston, Mass.

Foster, Adriance S., Dept. of Botany, University of California, Berkeley, Cal.

Fox, R. D., Bacteriological Labs., University of Akron, Akron, Ohio.

Genung, Elizabeth F., Burton Hall, Smith College, Northampton, Mass.

Guyer, M. F., Dept. of Zoology, University of Wisconsin, Madison, Wis.

Hale, Frank E., Mt. Prospect Laboratory, 355 Park Place, Brooklyn, N. Y.

Hance, Robert T., 1 Broadmoor Ave., Pittsburgh, 16, Pa.

Hartz, P. H., Curaçao, Dutch East Indies.

Hetherington, Duncan C., Dept. of Anatomy, Duke Hospital, Durham, N. C.

Hogue, Mary Jane, Dept. of Anatomy, University of Pennsylvania, Philadelphia, Pa.

Hood, E. G., Div. of Bacteriology & Dairy Research, Central Experimental Farm, Ottawa, Canada.

Hunter, Charles A., Kansas State Board of Health, National Reserve Bldg., Topeka, Kan.

Hutner, S. H., Haskins Laboratory, 305 E. 43rd St., New York 17, N. Y.

Jennison, M. W., Dept. of Biology, Mass. Institute of Technology, Cambridge, Mass.

Jordan, H. E., University of Virginia, Charlottesville, Va.

Levine, Max, Iowa State College, Ames, Ia.

Lippincott, Leon S., Stodder Laboratory, Eastern Maine General Hospital, Bangor, Me.

Macy, H., University Farm, St. Paul, Minn.

Mallmann, W. L., Dept. of Bacteriology, Michigan State College, East Lansing, Mich.

Marshall, M. S., Dept. of Bacteriology, University of California Medical School, San Francisco, Cal.

McClung, C. E., (University of Pennsylvania); 417 Harvard Ave., Swarthmore, Pa.

Miller, J. W., National Institute of Health, Bethesda, Md.

Myers, John T., Bendiner & Schlesinger Lab., Third Ave. & 10 St., New York, N. Y.

Painter, T. A., University of Texas, Austin, Texas.

Proescher, Frederick, 1344 University Ave., San Jose, Cal.

Randolph, L. F., Plant Science Bldg., Cornell University, Ithaca, N. Y.

Rohrbaugh, P. W., Research Dept., California Fruit Growers Exchange, 616 East Grove St., Ontario, Cal.

Scudder, S. A., City Hospital, Welfare Island, New York, N. Y.

Sharp, L. W., Cornell University, Ithaca, N. Y.

Shumway, Waldo, Dept. of Zoology, University of Illinois, Urbana, Ill.

Stearn, Mrs. E. W., Christian College, Columbia, Mo.

Steil, W. N., 1926 N. 53rd St., Milwaukee, Wis.

Stiles, Karl A., Coe College, Cedar Rapids, Ia.

Tanner, F. W., 362 Noyes Lab. of Chemistry, Urbana, Ill.

Taylor, Wm. Randolph, Dept. of Botany, University of Michigan, Ann Arbor, Mich.

Thomas, Stanley, Lehigh University, Bethlehem, Pa.

Thuringer, J. M., 801 N. E. 13th St., Oklahoma City, Okla.

Walton, Seth T., City Health Dept., Charlotte, N. C.

Ward, Henry B., (University of Illinois); Urbana, Ill.

Waterman, H. C., 3218 Stephenson Place, N. W., Washington, D. C.

Webster, R. C., Dept. of Anatomy, University of Louisville, Louisville, Ky.

Whitaker, Thomas W., Box 150, La Jolla, Cal.

Williams, B. G. R., Greencastle, Indiana.

Zirkle, Conway, Dept. of Botany, University of Pennsylvania, Philadelphia, Pa.